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New records of *Scleroderma* species (*Sclerodermataceae*, *Agaricomycetes*) from Pakistan

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Abstract — During a survey of gasteroid basidiomycetes from Pakistan, three *Scleroderma* species (*S. areolatum, S. chevalieri, S. dictyosporum*) have been identified that represent new records from Pakistan. A key to the known *Scleroderma* species from Pakistan is presented.

Key words — *Boletales*, earth balls, fungal taxonomy, Khyber Pakhtun Khwa, Nathia gali, Neelum valley

Introduction

Scleroderma Pers. is an extensively studied genus (Guzmán 1970, Sims et al. 1997, Kasuya et al. 2002, Baseia & Galvão 2002, Yoshimi 2000, 2002). This genus comprises 30 species worldwide, most of which occur in North America (Guzmán 1970, Kirk et al. 2008). *Scleroderma* species are thick-skinned earth balls that are separated primarily based on basidiospore characters (Sims et al. 1995). These gasteroid basidiomycetes form ectomycorrhizal associations with various trees and have a definite opening from which they eject their spores (Marshall 1904, Jeffries 1999).

Five *Scleroderma* species have been recorded previously from Pakistan (Ahmad et al. 1997). Continuing the survey of gasteroid basidiomycetes from Pakistan (Khalid & Iqbal 1996, 2004, Sultan et al. 2001, Razzaq & Shahzad 2004, 2007, Iqbal et al. 2006, Sultana et al. 2007, Moreno et al. 2009), we collected and identified three more *Scleroderma* species, which we describe here as an addition to the gasteroid mycota of Pakistan.

Materials & methods

Slides were prepared by mounting a smear of spores in a drop of 5% KOH, lactophenol and trypan blue. Spore ornamentation was observed under oil immersion (100×) objective. Spore size was determined by measuring the diameter of at least forty spores

including the ornamentation and then calculating the mean. Only mature spores were selected for the measurements. Peridium thicknesses, glebal color, surface ornamentation of basidiomata were also observed using 10× hand lens. Drawings were made with the help of camera lucida and measurements were taken using ocular micrometer.

Taxonomy

Scleroderma areolatum Ehrenb., Sylv. mycol. berol. 15: 27 (1818) Fig. 1

Basidiomata epigeous, ≤30 mm broad, 25 mm high, subglobose, attached to the substratum by a thick mycelial base, forming a pseudostipe; pseudostipe

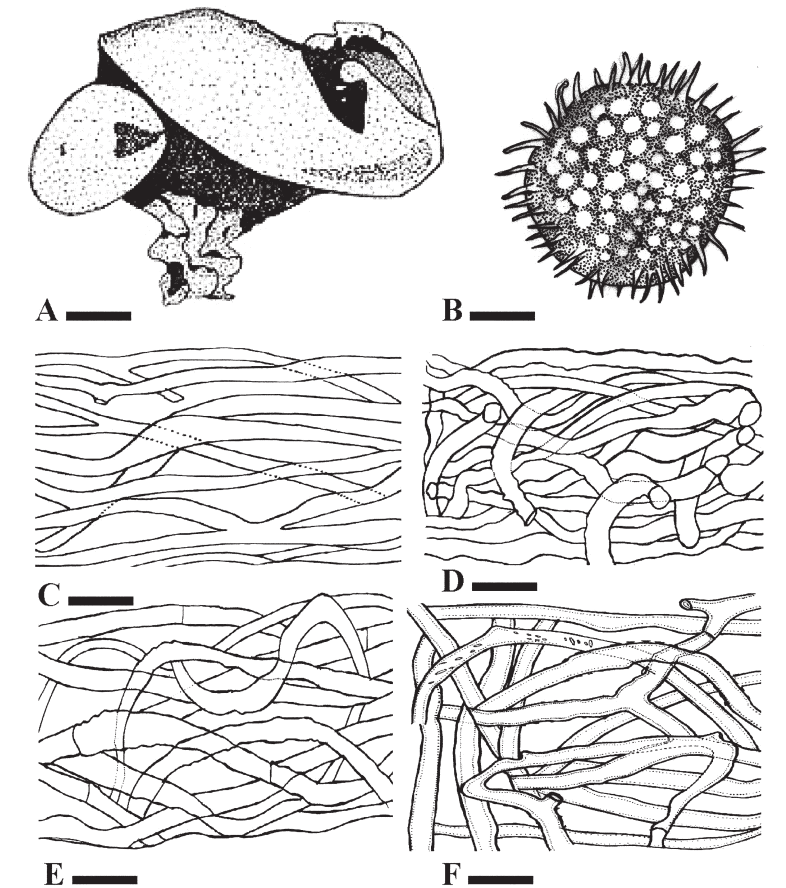

Fig. 1: *Scleroderma areolatum*. A. Basidiomata. B. Basidiospore. C. Exoperidial hyphae. D. Mesoperidial hyphae. E. Endoperidial hyphae. F. Tramal hyphae. Scale bars: A = 0.5 cm; B = 3.5 µm; C = 28 µm; D, F = 14 µm; E = 18 µm.

tough, often two basidiomata arise from the same tuft of mycelium. Rhizomorphs white, thick, growing individually or in small groups. Peridium thin, <1mm thick, pale yellow to brown; basal portion smooth, without scales, off white to pale yellow, upper part with brown spots like scales, dehiscence by tearing away the apical portion. Gleba powdery, dark purplish.

Basidiospores brown, globose, echinulate, never reticulate, 12–18 µm in diam., echines ≤2.5 µm long. Tramal Hyphae rarely septate, branched, hyaline, with few amorphous material encrusted, ≤5.5 µm wide, wall thickness ≤1 µm. Exoperidium composed of aseptate, branched hyphae, ≤7 µm wide, wall thickness ≤2.5 µm. Mesoperidium composed of aseptate, branched hyphae, wall encrusted, ≤7 µm wide, wall thickness 2.6 µm. Endoperidium composed of septate, branched hyphae, ≤6.6 µm wide, wall thickness ≤2.8 µm.

Specimen examined: **PAKISTAN**: **Khyber Pakhtun Khwa**, Khanspur, Ayubia, ca. 2575 m a.s.l., solitary on ground, in Himalayan moist temperate forest, 12 Aug. 2007, N. Yousaf NYG04 (LAH 120807).

Comments: The ectomycorrhizal *S. areolatum* is a poisonous species (Yamada & Katsuya 1995, Mason et al. 2000, Chen et al. 2006a,b) that is characterized by non-reticulate, densely echinulate basidiospores, a distinctive diagnostic feature, as other *Scleroderma* spp. examined have reticulate and sub-reticulate basidiospores. *Scleroderma areolatum* is widely distributed in Asia, Australia, Europe, North America, and South America (Lu et al. 1999, Giachini et al. 2000, Kasuya et al. 2002, Barroetaveña et al. 2005, Chen et al. 2006b).

Scleroderma chevalieri Guzmán, Ciencia Méx. 25: 202 (1967) Fig. 2

Basidioma epigeous, 45 mm broad, 40 mm high, subglobose, attached to the substratum via a tuft of mycelium, latter some time aggregated to form pseudostipe. Rhizomorphs white, thick. Peridium <1 mm thick, hard, rough, covered with patches of scales, mature specimen cracked at the upper portion, dehiscence by the irregular splitting of the peridium. Gleba pulverulent, pale yellow, brown to olivaceous.

Basidiospores brown, globose, echinulate, partially reticulate or sub reticulate, 11–17 µm in diam., echines ≤3 µm long. Tramal Hyphae aseptate, branched, hyaline, with clamp connections, ≤5.3 µm wide, wall thickness ≤1.3 µm. Exoperidium composed of septate, unbranched hyphae; ≤8.8 µm wide, wall thickness ≤2.0 µm. Mesoperidium composed of aseptate, unbranched hyphae, clamp connections rarely present; ≤9.5 µm wide, wall thickness ≤2.4 µm. Endoperidium composed of aseptate, unbranched hyphae, ≤9.5 µm wide, wall thickness 2.5 µm.

Specimen examined: **PAKISTAN**: **Khyber Pakhtun Khwa**, Nathia Gali, ca. 2501 m a.s.l., in groups, on ground, under *Abies pindrow* in Himalayan moist temperate forest, 25 Aug. 2006, N. Yousaf NYG03 (LAH 250806).

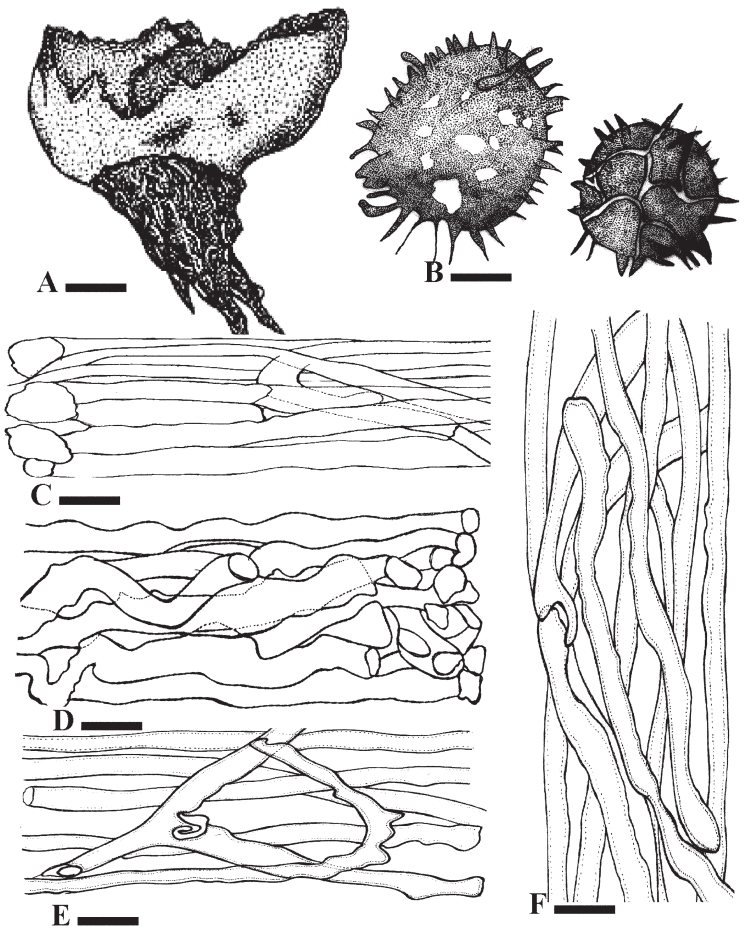

Fig. 2: *Scleroderma chevalieri*. A. Basidiome (weathered). B. Basidiospores. C. Exoperidial hyphae. D. Mesoperidial hyphae. E. Tramal hyphae. F. Endoperidial hyphae. Scale bars: A = 0.8 cm; B = 3.5 µm; C = 16 µm; D = 18 µm; E = 10 µm; F = 24 µm.

Comments: Like other members of *Scleroderma*, *S. chevalieri* has a hard, tough peridium and is characterized by a subglobose basidioma with short pseudostipe. It has large ornamented basidiospores with a sub-reticulum and peridium ≤1 mm thick. *Scleroderma sinnamariense* Mont. and *S. stellatum* Berk., also have a similar peridial thickness and partially reticulate spores. However, *S. sinnamariense* has a colored peridium with lemon yellow to black scales and smaller spores, and *S. stellatum* has a star-shaped opening and smaller spores (Sims et al. 1995).

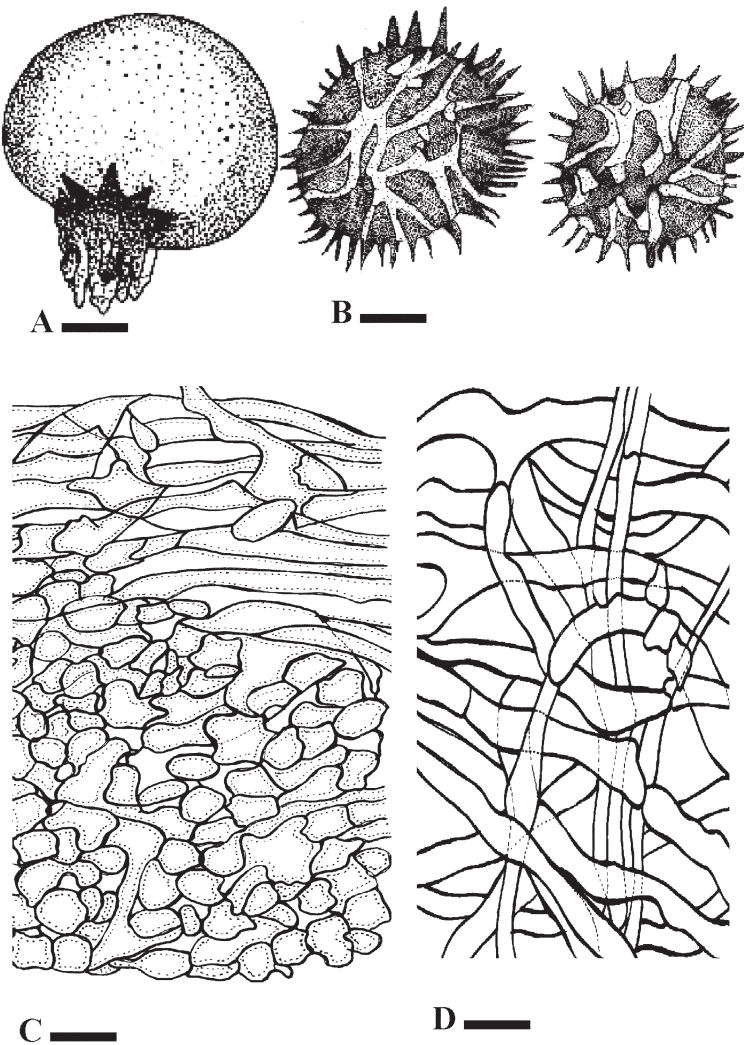

Fig. 3: *Scleroderma dictyosporum*. A. Basidiome. B. Basidiospores. C. Exoperidial and mesoperidial hyphae. D. Endoperidial hyphae. Scale bars: A = 0.6 cm; B = 3.5 µm; C = 28 µm; D = 10 µm.

Scleroderma dictyosporum Pat., Bull. Soc. Mycol. Fr. 12: 135 (1896) Fig. 3

Basidiomata epigeous, ≤30 mm broad and 30 mm high, globose to subglobose, dull brown, with well-developed mycelial base often produced into a pseudostipe; several fruit bodies arising from a single stalk. Peridium <1 mm thick, tough, relatively smooth, elastic, brittle in dried specimen cuticle

breaking into scales; scales black, thin, denser on top, spot-like; dehiscence by an irregular rupturing of an apical part. Gleba firm, compact, powdery with age, grayish to sepia, lighter towards the center.

Basidiospores brown, globose, echinulate-reticulate, 12–15 µm in diam., echines ≤4 µm long, young spores without reticulum, nurse cells prominent in the mount. Tramal hyphae hyaline, thin-walled, with clamp connections. Exoperidium composed of fascicles of erect hyphae, dark orange to brown, ≤6.5 µm, thick-walled, encrusted. Mesoperidium with globose elements, hyaline (yellowish in mass), ≤6 µm in diam., thin-walled, mixed with long hyaline hyphae. Endoperidium composed of septate, branched, hyaline hyphae, surrounding the gleba, hyphae ≤6 µm wide, thin-walled.

Specimen examined: **PAKISTAN**: **Khyber Pakhtun Khwa**, Naran (Kaghan valley), ca. 3350 m a.s.l., solitary on sandy soil, 23 Sep. 2009, N. Yousaf NYG01 (LAH 230909).

Comments: *Scleroderma dictyosporum* is an ectomycorrhizal species (Mukerji et al. 2000, Rai et al. 2009) and is distinguished by its globose basidioma with shorter pseudostipe, thin peridium (<1mm), echinulate-reticulate basidiospores (12.5–15.0 µm) with echines ≤4 µm. This species is close to *S. fuscum* (Corda) E. Fisch., but the latter has smooth peridium, absence of stipe and larger basidiospores (≤17 µm).

Key to *Scleroderma* species from Pakistan

1a. Basidiospores reticulate 2
1b. Basidiospores echinulate or verrucose, never reticulate 5

2a. Basidiospores partially to subreticulate 3
2b. Basidiospores completely reticulate 4

3a. Basidiospores 11–17 µm in diam., echines <3.0 µm long . . . . . *S. chevalieri*
3b. Basidiospores 5–8 µm in diam., echines <1µm long . . . . . *S. sinnamariense*

4a. Basidiospores 12–15 µm in diam., echines <4 µm long. . . . . *S. dictyosporum*
4b. Basidiospores 11–16 µm in diam., echines <1 µm long. . . . . *S. bovista*

5a. Basidiospores 8–13 µm. 6
5b. Basidiospores larger, 10–18 µm. 7

6a. Basidiospores 8–13 µm, echines >1 µm long. . . . . *S. verrucosum*
6b. Basidiospores 8.5–12 µm, echines <1 µm long . . . . . *S. cepa*

7a. Basidiospores 12–18 µm, echines >2.5 µm long . . . . . *S. areolatum*
7b. Basidiospores 10–14 µm, echines ≤1.5 µm long . . . . . *S. flavidum*

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